American Journal of Hematology 60:94–98 (1999)

Increased Truncated Form of Plasma Tissue Factor Pathway Inhibitor Levels in Patients With Disseminated Intravascular Coagulation

Minori Shimura,[1] Hideo Wada,[1,*] Takashi Nakasaki,[1] Katsuyo Hiyoyama,[1] Yositaka Mori,[2] Masakatsu Nishikawa,[1] Hiroshi Deguchi,[1] Katsumi Deguchi,[1] Esteban C. Gabazza,[3] and Hiroshi Shiku[1]

[1]Second Department of Internal Medicine, Mie University School of Medicine, Tsu-city, Japan
[2]Mie Red Cross Blood Center, Tsu-city, Japan
[3]Third Department of Internal Medicine, Mie University School of Medicine, Tsu-city, Japan

To evaluate that the relationship between the truncated form of tissue factor pathway inhibitor (TFPI) and the stage of disseminated intravascular coagulation (DIC), we measured the plasma levels of tissue factor (TF) antigen and the intact and truncated forms of TFPI antigens in 41 patients with DIC, 12 with pre-DIC, and 20 with non-DIC. The plasma TF and total TFPI antigen levels were significantly higher in patients with DIC than in non-DIC patients. Plasma levels of intact TFPI antigen in the pre-DIC groups were significantly lower than in the non-DIC and DIC groups. The truncated form of TFPI antigen levels in DIC patients were significantly increased compared with those in non-DIC and pre-DIC patients. The fact that the intact form of TFPI was decreased in pre-DIC patients compared with that in non-DIC patients, suggests that it is consumed in the pre-DIC state and that hypercoagulability occurs in pre-DIC patients. The increased level of the truncated form of TFPI in DIC patients may be attributed to proteolysis of the intact form of TFPI in these patients. The increased level of the truncated form of TFPI may be a useful index for the diagnosis of DIC. Am. J. Hematol. 60:94–98, 1999. © 1999 Wiley-Liss, Inc.

Key words: DIC; TFPI; TF; truncated form; intact form

INTRODUCTION

The tissue factor pathway inhibitor (TFPI), previously referred to as extrinsic pathway inhibitor [1] or lipoprotein-associated coagulation inhibitor (LACI) [2], is an endogenous anticoagulant protein of the serine protease inhibitor family. It is primary synthesized by the endothelium under normal physiologic conditions [3]. TFPI consists of three Kunitz-type inhibitory domains [4]; the second Kunitz domain is the FXa inhibitor, and the first domain is responsible for FVIIa/tissue factor (TF) inhibition [5]. The function of the third domain is not clear, but a segment including its C-terminal cationic tail is believed to be involved in binding to cell-surface glycosaminoglycans [6].

Disseminated intravascular coagulation (DIC) [7,8], a condition associated with severe bleeding tendency, organ failure, and sometimes with a very rapid and severe clinical course, is associated with vascular endothelial cell injury. Plasma TFPI levels have been reported to be within normal range or increased in patients with DIC in different studies [9,10]; they are increased in patients with systemic meningococcal disease [11]. Changes in the plasma TFPI level during the clinical course of DIC and its relationship with the prognosis of patients with DIC are still not clear. It was previously reported that both free TFPI (intact form) and truncated TFPI are pre-

Contract grant sponsor: Ministry of Education, Science, and Culture, Japan.

*Correspondence to: Hideo Wada, M.D., Second Department of Internal Medicine, Mie University School of Medicine, 2-174 Edobashi, Tsu-city, Mie-ken 514, Japan.

Received for publication 19 December 1997; Accepted 2 September 1998

TABLE I. Diagnostic Criteria for DIC*

		DIC score (points)
PT ratio	1.25–1.66	1
	>1.67	2
Fibrinogen (g/l)	1.00–1.50	1
	<1.00	2
FDP (μg/ml)	10–20	1
	20–40	2
	>40	3
Organ failure due to thrombosis	(+)	1

*DIC, disseminated intravascular coagulation; PT, prothrombin time; FDP, fibrin/fibrinogen degradation products. The sum of the DIC Score was 4 or higher.

TABLE II. Subjects*

	DIC	Pre-DIC	Non-DIC
APL	8	1	3
AML	6	3	5
AMMoL	2	1	3
CML,bc	5	1	1
ALL	6	2	3
MDS	2	0	1
NHL	9	2	3
Others	3	2	1
Total	41	12	20

*DIC, disseminated intravascular coagulation; APL, promyelocytic leukemia; AML, acute myeloblastic leukemia; AMMoL, acute myelomonocytic leukemia; CML,bc, chronic myelocytic leukemia with blastic crisis; ALL, acute lymphoblastic leukemia; MDS, myelodysplasia; NHL, non-Hodgkin's lymphoma.

sent in plasma, and that the truncated form of TFPI has less anticoagulant activity compared with its intact form [12,13].

In this study, we evaluated the sequential changes in the plasma levels of total TFPI, free TFPI, and truncated TFPI during the clinical course of patients with DIC, pre-DIC, or non-DIC.

MATERIALS AND METHODS

Subjects

This study comprised 41 patients with DIC, 12 with pre-DIC, and 20 without DIC (non-DIC group). The diagnosis of DIC was based on the criteria established by the Japanese Ministry of Health and Welfare (Table I) [14,15]. In 12 DIC patients, a hemostatic test performed the previous week allowed their classification in the ''pre-DIC'' group [16]. The underlying diseases of patients with DIC were acute myeloblastic leukemia (AML), 11 patients; acute promyelocytic leukemia (APL), 11 patients; acute myelomonocytic leukemia (AMMoL), 5 patients; chronic myelocytic leukemia with blastic crisis (CML,bc), 6 patients; acute lymphoblastic leukemia (ALL), 9 patients; malignant lymphoma stage IV, 12 patients; myelodysplastic syndrome, 3 patients; and other diseases, 4 patients (Table II). DIC patients were treated with only gabexate mesilate (FOY), a synthetic proteinase inhibitor [17,18] that inhibits the activity of thrombin, factor Xa, plasmin, and plasma kallikrein; none of the patients received heparin. The efficacy of the DIC treatment was assessed after seven days using the DIC scoring system shown in Table I. The outcome was defined as good if the DIC score and the symptoms improved and the patient survived; the poor outcome was defined as an increase in the DIC score, worsening of symptoms, or death of the patient.

Plasma TF antigen levels were measured using an IMUBIND Tissue Factor enzyme-linked immunosorbent assay (ELISA) kit (ADI, Greenwich, CT). The samples were diluted 1:10 in 0.05 M Tris, pH 7.5, with 2% bovine serum albumin and 0.05% Tween 20 buffer to eliminate the matrix effect of undiluted plasma. The monoclonal antibody of this kit is a murine immunoglobulin G1 against human brain tissue factor; it detects the TF-apoprotein complex, TF, and TF-FVII complex [19,20]. The plasma total TFPI level was measured using an IMUBIND® Total TFPI ELISA kit (ADI) [21]. The TFPI antigens in plasma samples were captured in microtest wells precoated with anti-TFPI polyclonal antibody. Plasma TFPI antigens were detected using a biotinylated monoclonal antibody specific for the Kunitz domain 1 of TFPI. This kit measures native, complexed, and truncated TFPI. The plasma free-TFPI antigens were measured with an IMUBIND® Free TFPI ELISA kit (ADI). TFPI antigens in plasma samples were captured in microtest wells precoated with anti-TFPI polyclonal antibody. The free-TFPI antigens were detected using a biotinylated monoclonal antibody specific for the Kunitz domain 3 of TFPI. The plasma truncated-TFPI levels were calculated as the difference between the total-TFPI and the free-TFPI values.

Data are expressed as the means ± standard deviation. Statistical difference between two groups was assessed by Wilcoxon's nonpaired test, and among three groups of ANOVA.

RESULTS

Among the DIC, pre-DIC, and non-DIC groups, the plasma TF and total-TFPI antigen levels were significantly increased in the DIC group compared with levels observed in the other two groups ($p < 0.01$). In pre-DIC patients, the plasma TF antigen and total-TFPI antigen levels were not significantly increased compared with those in the non-DIC patients, but the TF/total TFPI ratio was significantly higher than the ratio observed in the other two groups ($P < 0.05$) (Table III). The intact form of TFPI antigen level in pre-DIC patients was significantly lower than those in pre-DIC and non-DIC groups

TABLE III. Plasma Levels of TF and TFPI in Non-DIC, Pre-DIC, and DIC Patients†

	TF (pg/ml)	Total TFPI (ng/ml)	TF/total TFPI ($\times 10^{-3}$
Non-DIC	204.0 ± 49.2	198.8 ± 41.2	1.12 ± 0.52
Pre-DIC	238.1 ± 68.6	163.3 ± 61.5	5.13 ± 1.84*
DIC	288.9 ± 77.6**	301.4 ± 87.6**	3.08 ± 1.38

†TF, tissue factor; TFPI, tissue factor pathway inhibitor; DIC, disseminated intravascular coagulation.
*$P < 0.05$.
**$P < 0.01$.

TABLE IV. Plasma Level of Various TFPI Forms in Non-DIC, Pre-DIC, and DIC† Patients

	Total TFPI (ng/ml)	Intact TFPI (ng/ml)	Truncated TFPI (ng/ml)	Intact/ truncated ratio
Non-DIC	198.8 ± 41.2	84.8 ± 24.7	114.5 ± 32.0	0.79 ± 0.29
Pre-DIC	163.3 ± 61.5	47.5 ± 15.6*	115.8 ± 60.9	0.48 ± 0.21**
DIC	301.4 ± 87.6*	103.6 ± 32.7	195.1 ± 82.9*	0.61 ± 0.27

†TFPI, tissue factor pathway inhibitor; DIC, disseminated intravascular coagulation.
*$P < 0.01$.
**$P < 0.05$.

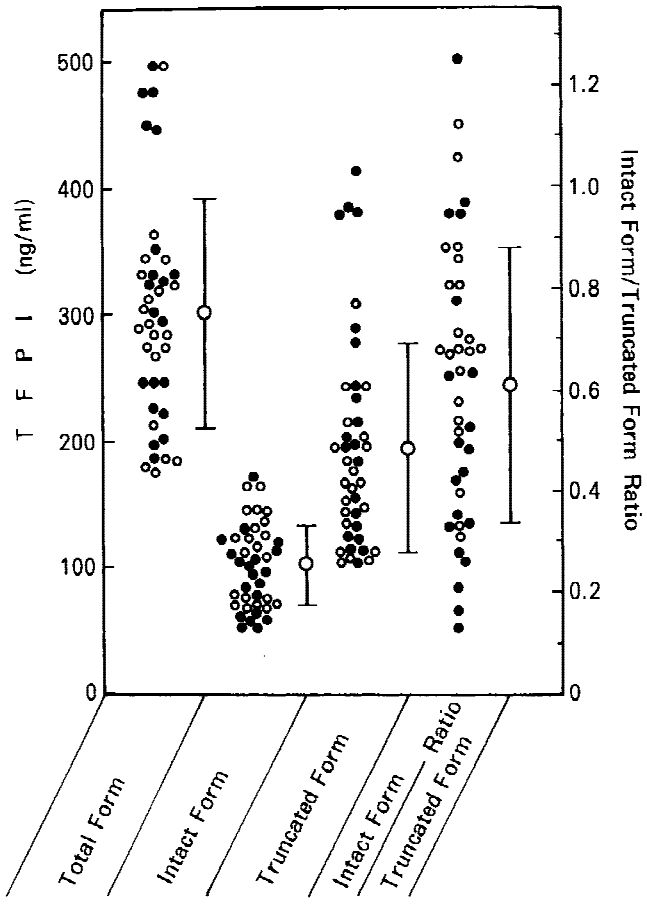

Fig. 1. Various forms of TFPI antigen in the plasma of DIC patients. ○, good outcome; ●, poor outcome. Circles with vertical bars represent the mean ± SD.

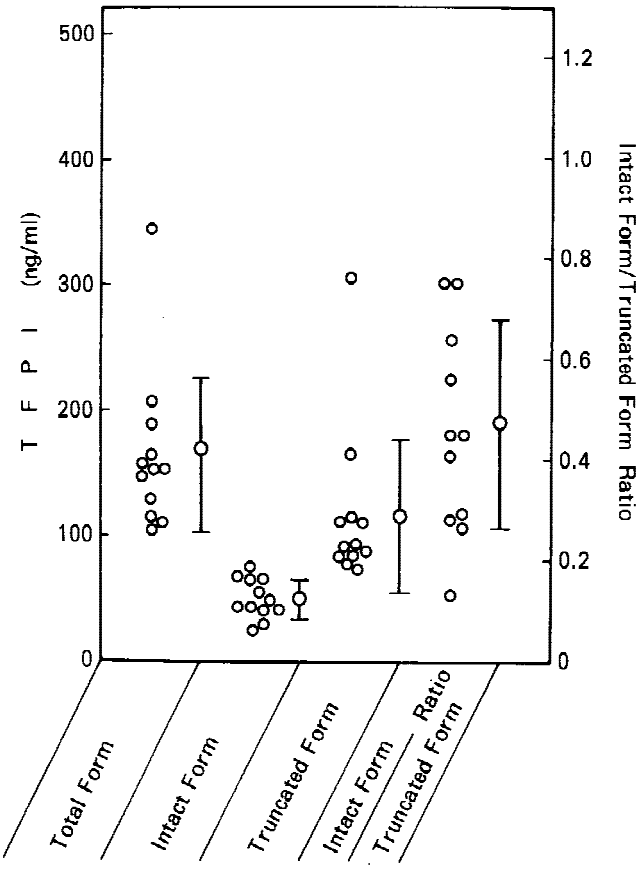

Fig. 2. Various forms of TFPI antigen in the plasma of pre-DIC patients.

($P < 0.01$). The truncated form of TFPI levels in the DIC group were significantly increased as compared with levels in other groups ($P < 0.01$). The plasma intact form of TFPI/truncated form of TFPI ratio was significantly lower in pre-DIC patients than in those of the DIC and non-DIC groups (Table IV). In DIC patients, the plasma total TFPI was significantly increased as compared with non-DIC patients; the levels of the truncated form of TFPI were particularly high. There was no significant difference in the plasma TF or total-TFPI levels between the DIC patients with a good outcome and those with a poor outcome. The intact form of TFPI and the ratio of intact form/truncated form were decreased in patients with poor outcome (Fig. 1). Plasma level of total TFPI was slightly decreased (due to significant decrease of intact form of TFPI) in the pre-DIC group (Fig. 2), compared with levels in the non-DIC group (Fig. 3). The TF/total-TFPI and the TF/free-TFPI ratio were higher in pre-DIC patients than in the other two groups (Fig. 4).

DISCUSSION

The plasma levels of TF and total-TFPI antigen were significantly increased in patients with DIC compared

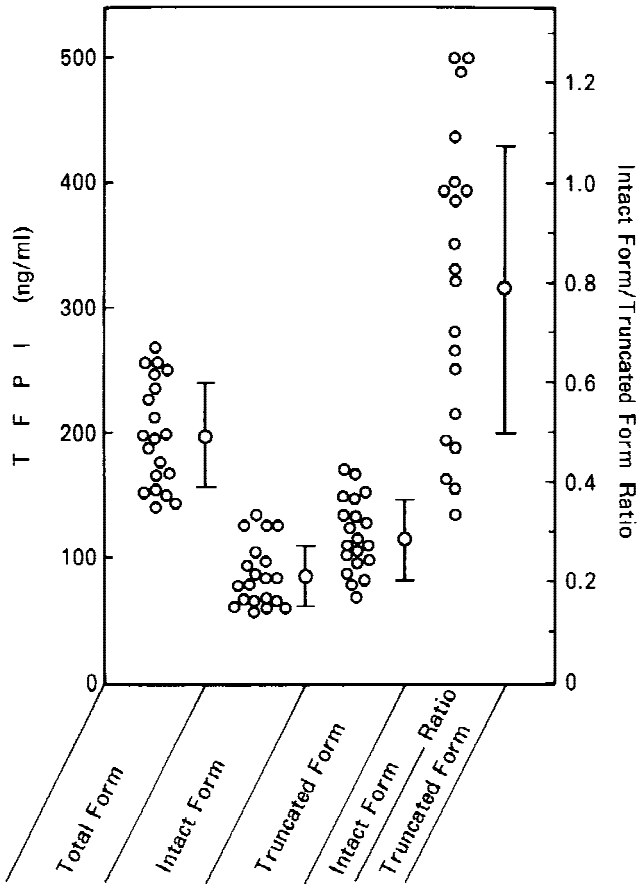

Fig. 3. Various forms of TFPI antigen in the plasma of non-DIC patients.

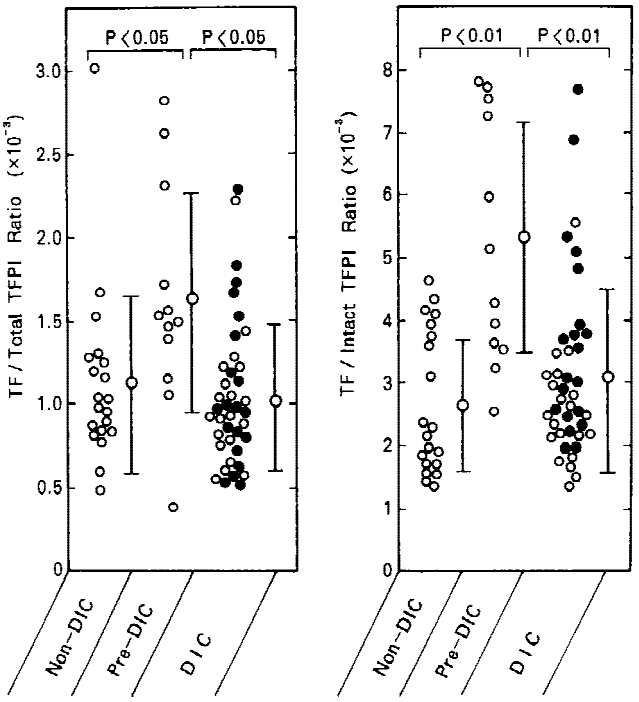

Fig. 4. TF/total-TFPI and TF/intact-TFPI ratios in the non-DIC, pre-DIC, or DIC patients. ○, good outcome; ●, poor outcome.

with those without DIC. Since TF is the major initiator of DIC [8,22,23], the presence of high TF levels in plasma indicates a pathologic state. TFPI, which inhibits the TF pathway, is considered to play important roles in various hypercoagulable states. It was reported that plasma TFPI activity in patients with DIC is increased or normal and that this activity is not decreased in DIC patients with severe liver disorder [24]. Plasma TFPI is almost exclusively produced in vascular endothelial cells, and it may be present on these cells bound to glycosaminoglycans [25,26]. We previously found that plasma TF level is significantly increased in both DIC and pre-DIC patients, that the plasma TFPI level is significantly increased in DIC patients, but that this latter is not markedly increased in pre-DIC patients [10]. In our present investigation, the level of the intact form of TFPI was increased in DIC patients compared with that in pre-DIC patients, suggesting that the intact form of TFPI is released from vascular endothelial cells after the onset of DIC. Because glycosaminoglycan on endothelial cells are decreased in DIC, the intact TFPI cannot bind to these cells. It was reported that the carboxyterminal region mediates TFPI binding to cell surface [27]. In DIC, some proteases may cleave to this site of TFPI, and TFPI lacking the carboxy-terminal may then be released from vascular endothelial cells. The truncated form of TFPI levels in DIC patients were significantly increased compared with pre-DIC and non-DIC patients. Increased conversion of intact to truncated form of TFPI is considered to occur in DIC patients. These findings indicate that both the release of intact form of TFPI and the conversion of the intact form to the truncated form of TFPI are significantly increased in DIC patients. The levels of the intact form of TFPI antigen were lower in pre-DIC patients than in the other groups of patients. The fact that the plasma level of the truncated form of TFPI was similar in pre-DIC and non-DIC patients, suggests that the intact form of TFPI is consumed in the pre-DIC state. The plasma intact form/truncated form ratio of TFPI was significantly decreased in pre-DIC patients compared with the other groups, suggesting that a marked hypercoagulability occurs in pre-DIC patients and that it worsens with the progression of the diseases. There was no significant difference in the plasma levels of TF and total TFPI between DIC patients with good outcome and those with poor outcome. However, in patients with poor outcome and DIC, the TF/total TFPI ratio tended to be high, and the TF/intact TFPI ratio was significantly increased. Decreased plasma TFPI levels have been reported in patients with thrombotic thrombocytopenic purpura (TTP) [21]; patients with TTP have increased plasma levels of thrombomodulin resulting from a severe systemic vascular endothelial injury [14,21]. The intact form of TFPI in plasma is considered

to derive mainly from vascular endothelial cells—a severe systemic vascular endothelial injury may explain the reduction in the plasma level of the intact form of TFPI; this phenomenon may also explain the poor outcome in patients with DIC or TTP. The ratio of TF/intact TFPI was inversely correlated to the severity of the disease. In the present DIC group, poor outcome was associated with a low release of TFPI from vascular endothelial cells and with a higher TF/intact-TFPI ratio.

In conclusion, measurement of the intact form and truncated forms of TFPI may be important for assessing the clinical course of DIC.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science, and Culture, Japan.

REFERENCES

1. Rapaport SI. The extrinsic pathway inhibitor: A regulation of tissue type factor-dependent blood coagulation. Thromb Haemost 1991;66: 6–15.
2. Broze GJ, Warren LA, Novotny WF, Higuchi DA, Girard TJ, Miletich JP. The lipoprotein-associated coagulation inhibitor that inhibits factor VII-tissue factor complex also inhibits Xa: Insight into its possible mechanism of action. Blood 1988;71:335–343.
3. Bajaj MS, Kuppuswamy MN, Saito H, Spitzer SG, Bajaj SP. Cultured normal human hepatocytes do not synthesize lipoprotein-associated coagulation inhibitor: Evidence that endothelium is the principal site of its synthesis. Proc Natl Acad Sci USA 1990;87:8869–8873.
4. Wun TC, Kretzmer KK, Girard TJ, Miletich JP, Broze GJ. Cloning and characterization of a cDNA coding for the lipoprotein associated coagulation inhibitor shows that it consists of three tandem Kunitz type inhibitory domains. J Biol Chem 1988;263:6001–6004.
5. Girard TJ, Warren LA, Novotny WF, Likert KM, Brown SG, Meletich JP, Broze GJ. Functional significance of the Kunitz type inhibitory domains of lipoprotein associated coagulation inhibitor. Nature 1989; 338:518–520.
6. Enjyoji K, Miyata T, Kamikubo Y, Kato H. Effect of heparin on the inhibition of factor Xa by tissue factor pathway inhibitor: A segment, Gly 212-Phe 243, of the third Kunitz domain is a heparin binding site. Biochemistry 1995;34:5725–5735.
7. Müller-Berghaus G. Pathophysiologic and biochemical events in disseminated intravascular coagulation: Dysregulation of procoagulant and anticoagulant pathways. Semin Thromb Haemost 1989;15:58–98.
8. Wada H, Ohiwa M, Kaneko T, Tamaki S, Tanigawa M, Shirakawa S, Koyama M, Hayashi T, Suzuki K. Plasma thrombomodulin as a marker of vascular disorders in thrombotic thrombocytopenic purpura and disseminated intravascular coagulation. Am J Hematol 1992;39: 20–24.
9. Weissbach G, Herenberg J, Wendisch J, Pargec N, Thomas K. Tissue factor pathway inhibitor in infants and children. Thromb Res 1994; 73:441–446.
10. Shimura M, Wada H, Wakita Y, Nakase T, Hiyoyama K, Nagaya S, Mori Y, Shiku H. Plasma tissue factor pathway inhibitor levels in patients with disseminated intravascular coagulation. Am J Hematol 1996;52:165–170.
11. Brandtzaeg P, Sandset PM, Joø GB, Øvstevø R, Abildgaard U, Kierulf P. The quantitative association of plasma endotoxin, antithrombin, protein C, extrinsic pathway inhibitor and fibrinopeptide A in systemic meningcoccal disease. Thromb Res 1989;55:459–470.
12. Broze GJ Jr. Tissue factor pathway inhibitor. Thromb Haemost 1995; 74:90–93.
13. Nordfang O, Bjorn SE, Valantin S, Nielsen LS, Wildgoose P, Beck TC, Hedner U. The C-terminal of tissue factor pathway inhibitor is essential to its anticoagulant activity. Biochemistry 1991;30:10371–10376.
14. Wada H, Minamikawa K, Wakita Y, Nakase T, Ohiwa M, Tamaki S, Deguchi K, Shirakawa S, Hayashi T, Suzuki K. Increased vascular endothelial cell markers in patients with disseminated intravascular coagulation. Am J Hematol 1993;44:85–88.
15. Kobayashi N, Maegawa T, Takada M, Tanaka H, Gonmori H. Criteria for diagnosis of DIC based on the analysis of clinical and laboratory findings in 345 DIC patients collected by the Research Committee on DIC in Japan. Bibl Haemotol 1987;49:265–275.
16. Wada H. Minamikawa K. Wakita Y, Nakase T, Ohiwa M, Tamaki S, Deguchi A, Mori Y, Deguchi K, Shirakawa S, Suzuki K. Hemostatic study before onset of disseminated intravascular coagulation. Am J Hematol 1994;43:190–194.
17. Ohno H, Kosaki G, Kambayashi J, Imaoka S, Hirata F. FOY; [ethyl p-(6-guanidinohexanoxyloxy) benzoate] methanesulfonate as a serine protease inhibitor. I. Inhibition of thrombin and factor Xa in vitro. Thromb Res 1980;19:579–588.
18. Ohno H, Kambayashi J, Chang SW, Kosaki G. FOY; [ethyl p-(6-guanidinohexanoxyloxy) benzoate] methanesulfonate as a serine protease inhibitor. II. In vivo effect on coagulofibrinolytic system in comparison with heparin or aprotinin. Thromb Res 1981;24:445–452.
19. Morrissey JH, Fair DS, Edgington TS. Monoclonal antibody analysis of purified and cell-associated tissue factor. Thromb Res 1988;52:247–261.
20. Wada H, Nakase T, Nakaya R, Minamikawa K, Wakita Y, Kaneko T, Ohiwa M, Deguchi K, Shirakawa S. Elevated plasma tissue factor antigen level in patients with disseminated intravascular coagulation. Am J Hematol 1994;45:232–236.
21. Kobayashi M, Wada H, Wakita Y, Shimura M, Nakase T, Hiyoyama K, Nagaya S, Minami N, Nakano T, Shiku H. Decreased plasma tissue factor pathway inhibitor levels in patients with thrombotic thromocytopenia purpura. Thromb Hemost 1995;73:10–14.
22. Gralnick HR, Abrell E. Studies on the procoagulant and fibrinolytic activity of promyelocytic leukemia. Br J Haematol 1973;24:89–98.
23. Wada H, Nagano T, Tomeoku M, Kuto M, Karitani Y, Deguchi K, Shirakawa S. Coagulent and fibrinolytic activities in the leukemic cell lysates. Thromb Res 1982;30:315–322.
24. Warr TA, Rao LVM, Rapaport SI. Human plasma extrinsic pathway inhibitor activity; II plasma levels in disseminated intravascular coagulation and hepatocellular disease. Blood 1989;74:994–998.
25. Sandset PM, Høgevold HE, Lyberg T, Andersson TR, Abildgaard U. Extrinsic pathway inhibitor in elective surgery; A comparison with other coagulation inhibitors. Thromb Haemostas 1989;62:856–860.
26. Novotny WF, Palmier M, Wun T-C, Broze GJ, Miletich JP. Purification and properties of heparin-releasable lipoprotein-associated coagulation inhibitor. Blood 1991;78:394–400.
27. Warshawsky I, Bu G, Mast A, Saffitz JE, Broze GJ Jr, Schwartz AL. The carboxy terminus of tissue factor pathway inhibitor is required for interacting with hepatoma cells in vitro and in vivo. J Clin Invest 1995;95:1773–1781.